

CLAIMS

1. A system for measuring the irradiance of a fluorescently labeled particle, comprising:

a cytometric flow chamber having a flow path for passage of the fluorescently labeled particle;

a plurality of excitation light sources, each emitting a beam of light incident on the cytometric flow chamber;

a plurality of scatter detectors in optical communication with the flow path of the cytometric flow chamber, each configured to detect light from only one of the plurality of excitation light sources and arranged so as to detect scattered light from the fluorescently labeled particle as it passes through the flow path of the cytometric flow chamber;

a trigger connected to the plurality of scatter detectors, the trigger emitting a signal when scattered light incident on one of the scatter detectors is exceeding a predetermined threshold value;

a collection optics in optical communication with the flow path of the cytometric flow chamber to collect emissions from the fluorescently labeled particle;

at least one fluorescence detector to receive the emissions collected by the collection optics and generate an output, the at least one fluorescence detector being configured to respond only to a discrete number of wavelength bands; and

at least one integrator connected to the trigger and the at least one fluorescence detector, for recording the output of the at least one fluorescence detector in response to a signal from the trigger.

2. The system of claim 1 further comprising three excitation light sources and three scatter detectors, each scatter detector being configured to detect light from only one of the three excitation light sources.

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3. The system of claim 2 wherein the three excitation light sources are configured along the flow path; and wherein the three scatter detectors are arranged with each scatter detector corresponding to a different one of the three excitation light sources.

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4. The system of claim 3 wherein a bandpass filter is positioned in front of each scatter detector allowing only one of the three excitation light wavelengths to reach the detector.

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5. The system of claim 2 wherein:

the three excitation light sources are positioned about an excitation light axis;

a fiber optic bundle is configured around the excitation light axis, the fiber optic bundle containing three sets of optical fibers; and

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each set of optical fibers is optically coupled to a different one of the three scatter detectors.

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6. The system of claim 1 wherein each of the plurality of scatter detectors further comprises a bandpass filter, each bandpass filter allowing light from only one excitation light source to pass through.

7. The system of claim 1 wherein at least two excitation light sources are focused to overlap in the flow path of the flow chamber.

5 8. The system of claim 1 wherein each of the plurality of scatter detectors further comprises a photodiode.

9. The system of claim 8 wherein the at least one fluorescence detector comprises a photomultiplier tube.

10 10. The system of claim 9 wherein the at least one fluorescence detector comprises filter having at least one bandpass.

15 11. The system of claim 9 wherein at least one of the plurality of excitation light sources comprises a laser.

12. A system for measuring the irradiance of a fluorescently labeled particle, comprising:

20 a cytometric flow chamber having a flow path for passage of the fluorescently labeled particle;

two lasers, each laser emitting a beam of light incident on the cytometric flow chamber;

25 two scatter sensors in optical communication with the flow path of the cytometric flow chamber, each scatter sensor being configured to detect light from only one of the two lasers and arranged so as to detect scattered light from the fluorescently labeled particle as it passes through the flow path of the cytometric flow chamber;

a trigger connected to the two photodiodes, the trigger emitting a signal when scattered light incident on one of the scatter sensors is exceeding a predetermined threshold value;

5 a collection optics in optical communication with the flow path of the cytometric flow chamber to collect emissions from the fluorescently labeled particle;

at least one photomultiplier tube to receive the emissions collected by the collection optics and generate an output, the at least one photomultiplier tube being configured to respond to
10 only two wavelength bands; and

at least one integrator connected to the trigger and the at least one fluorescence detector, for recording the output of the at least one fluorescence detector in response to a signal from the trigger.

15 13. A system for measuring the irradiance of a fluorescently labeled particle, comprising:

a plurality of excitation light sources, each emitting a beam of light incident on the fluorescently labeled particle;

20 a plurality of scatter detectors, each configured to detect light from only one of the plurality of excitation light sources and arranged so as to detect scattered light from the fluorescently labeled particle as the fluorescently labeled particle is illuminated by one of the plurality of excitation
25 light sources;

a trigger connected to the plurality of scatter detectors, the trigger emitting a signal when scattered light incident on one of the scatter detectors is exceeding a predetermined threshold value;

at least one fluorescence detector to collect emissions from the fluorescently labeled particle and generate an output, the at least one fluorescence detector being configured to respond only to a discrete number of wavelength bands; and

5 at least one integrator connected to the trigger and the plurality of fluorescence detectors, for recording the output of at least one of the plurality of fluorescence detectors in response to a signal from the trigger.

10 14. A system for measuring the fluorescence of a particle having a plurality of dyes, the system comprising:

a) a predetermined number of lasers, at least one laser exciting a maximum number of dyes;

15 b) at least one fluorescence detector, each fluorescence detector further comprising:

(i) a photomultiplier tube; and

(ii) a multi-bandpass filter in optical communication with the photomultiplier tube, the multi-bandpass filter passing a number of discrete wavelength bands to the photomultiplier tube, the number being less than or equal to the predetermined number of lasers; and

20 wherein the wherein the number of fluorescence detectors is equal to the maximum number of dyes.

25 15. The system of claim 14 further comprising:

c) a plurality of scatter detectors equal to the number of lasers;

30 d) a trigger coupled to the scatter detectors, the trigger emitting a signal when scattered light incident on one of the scatter detectors exceeds a predetermined threshold value; and

e) at least one integrator coupled to the trigger and to the at least one fluorescence detector, the integrator recording the output of the at least one fluorescence detector in response to a signal from the trigger.

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16. A method for measuring the fluorescence of a particle having a plurality of dyes, the method comprising:

a) interrogating a particle with a first excitation light source;

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b) detecting the interrogation of the particle with the first excitation light source using a scatter detector configured to only detect light from the first excitation light source;

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c) detecting any fluorescence emitted by the particle using a fluorescence detector when it is detected that the particle is being interrogated by the first excitation light source;

d) interrogating a particle with a second excitation light source;

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e) detecting the interrogation of the particle with the second excitation light source using a scatter detector configured to only detect light from the second excitation light source; and

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f) detecting any fluorescence emitted by the particle using a fluorescence detector when it is detected that the particle is being interrogated by the second excitation light source.

17. A method for measuring the fluorescence of a particle having a plurality of dyes, the method comprising:

a) interrogating a particle with a first excitation light source;

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b) detecting the interrogation of the particle with the first excitation light source using a scatter detector configured to only detect light from the first excitation light source;

5 c) detecting any fluorescence emitted by the particle using a plurality of fluorescence detectors when it is detected that the particle is being interrogated by the first excitation light source;

10 d) assigning any detected fluorescence to dyes known to be excited by the first excitation light source;

e) interrogating a particle with a second excitation light source;

15 f) detecting the interrogation of the particle with the second excitation light source using a scatter detector configured to only detect light from the second excitation light source;

20 g) detecting any fluorescence emitted by the particle using the plurality of fluorescence detectors when it is detected that the particle is being interrogated by the second excitation light source, the number of fluorescence detectors being equal to the larger of the maximum number of dyes stimulated by the first excitation light source and the maximum number of dyes stimulated by the second excitation light source; and

25 h) assigning any detected fluorescence to dyes known to be excited by the second excitation light source.

18. The method of claim 17 wherein at least one of the plurality of fluorescence detectors comprises a filter that only passes light emitted by a first dye upon excitation by the first

excitation light source and light emitted by a second dye upon excitation by the second excitation light source.

19. A system for measuring the irradiance of a
5 fluorescently labeled particle, comprising:

a cytometric flow chamber having a flow path for passage of the fluorescently labeled particle;

a plurality of excitation light sources, each emitting a beam of light incident on the cytometric flow chamber;

10 a plurality of scatter detectors in optical communication with the flow path of the cytometric flow chamber, each configured to detect light from only one of the plurality of excitation light sources and arranged so as to detect scattered light from the fluorescently labeled particle as it passes
15 through the flow path of the cytometric flow chamber;

a plurality of triggers, each of the plurality of triggers being coupled to a separate corresponding one of the plurality of scatter detectors, and each trigger emitting a signal when scattered light incident on the corresponding scatter detector
20 exceeds a predetermined threshold value;

collection optics in optical communication with the flow path of the cytometric flow chamber to collect emissions from the fluorescently labeled particle;

25 a plurality of fluorescence detectors to receive the emissions collected by the collection optics and generate an output, each of the fluorescence detectors being configured to respond only to a discrete number of wavelength bands; and

a plurality of integrators, each integrator being coupled to a separate corresponding one of the plurality of triggers, and each integrator being configured to record the output of at
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least one of the plurality of fluorescence detectors in response to a signal from the corresponding trigger.

20. The system of claim 19 further comprising a controller
5 coupled to the plurality of integrators and the plurality of
triggers, the controller being programmed to control the
plurality of integrators and the plurality of triggers to
prevent anomalous data from being acquired.

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